Identification of a Ckit+ Colonic Crypt Base Secretory Cell that Supports Lgr5+ Stem Cells in Mice.

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Public Summary:
The lining of the colon is continuously replaced throughout our lives. This process is driven by self-renewing Lgr5+ stem cells in the colonic epithelium. The regulation of these cells is likely to be important in normal physiology as well as in diseases such as colitis and colon cancer. These stem cells require a specialized “niche” to regulate their growth—a local microenvironment consisting of adjacent cells, regulatory factors, and extracellular matrix. Recently, it was shown that Lgr5+ stem cells in the small intestine are regulated by adjacent Paneth cells; thus, Paneth cells are niche cells for small intestinal stem cells. However, no such niche cell has been identified in the colon. In this study, we used multicolor flow cytometry to isolate and characterize cells from distinct regions of the colonic epithelium. We used immunostaining, single cell gene expression analysis, and in-vivo and in-vitro functional assays to define four major epithelial subtypes or transcriptional states in select populations of colon epithelial cells. Within one of these subtypes (the goblet cells), we observed a population that represents a novel colonic niche cell. These cells express the surface marker cKit/CD117+, express several growth factors known to be important in colon stem cell regulation, and promote the in-vitro growth of stem cells. Interestingly, we also found that cKit labels Paneth cells in the small intestine. Thus, in the colon, cKit labels a subset of goblet cells that resembles small intestinal Paneth cells and contributes to the colonic Lgr5+ stem cell niche.

Scientific Abstract:
BACKGROUND & AIMS: Paneth cells contribute to the small intestinal niche of Lgr5+ stem cells. Although the colon also contains Lgr5+ stem cells, it does not contain Paneth cells. We investigated the existence of colonic Paneth-like cells that have a distinct transcriptional signature and support Lgr5+ stem cells. METHODS: We used multicolor fluorescence-activated cell sorting (FACS) to isolate different subregions of colon crypts, based on known markers, from dissociated colonic epithelium of mice. We performed multiplexed single-cell gene expression analysis with quantitative reverse transcriptase PCR, followed by hierarchical clustering analysis, to characterize distinct cell types. We used immunostaining and FACS analyses, along with in vivo administration of a Notch inhibitor and in vitro organoid cultures, to characterize different cell types. RESULTS: Multicolor FACS could isolate distinct regions of colonic crypts. Four major epithelial subtypes or transcriptional states were revealed by gene expression analysis of selected populations of single cells. One of these, the goblet cells, contained a distinct cKit/CD117+ crypt base subpopulation that expressed Dll1, Dll4, and epidermal growth factor, similar to Paneth cells, which were also marked by cKit. In the colon, cKit+ goblet cells were interdigitated with Lgr5+ stem cells. In vivo, this colonic cKit+ population was regulated by Notch signaling; administration of a gamma-secretase inhibitor to mice increased the number of cKit+ cells. When isolated from mouse colon, cKit+ cells promoted formation of organoids from Lgr5+ stem cells, which expressed Kitl/SCF, the ligand for cKit. When organoids were depleted of cKit+ cells using a toxin-conjugated antibody, organoid formation decreased. CONCLUSIONS: cKit marks small intestinal Paneth cells and a subset of colonic goblet cells that are regulated by Notch signaling and support Lgr5+ stem cells.